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TITLE: Role of Non-neuronal Cells in Tauopathies After Brain Injury

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14. ABSTRACT

The main purpose of this study is to identify how, after mild repeated traumatic brain injury, specific inflammatory factors (components of the complement cascade) elevated during long asymptomatic prodromal period are responsible for the eventual onset of cognitive deficits and neurodegeneration. We investigate how inflammation leads to accumulation of aberrant tau aggregates, a common downstream pathway directly causing neurodegeneration in many neurodegenerative disease, including TBI. We use a human Tau Tg mouse with its native promoter to model effects of injury on normal tau expression, an unstudied response that may be critical to understanding this elusive delay in onset of symptoms. This mouse is bred to mice with novel transgenes associated with complement activation: one lacking the "brake" of the complement cascade (C1inh KO) and the other overexpressing C5a. During this first year period we have produced new data from an old experiment that provides exciting new information related to this proposal. We show that C1q alone can cause presynaptic deficits and aberrant inflammation, and that the presence of subtoxic levels of beta-amyloid cause vulnerability to C1q, leading to large increases in neuronal tau accumulation corresponding to postsynaptic and cognitive deficits, and importantly that these adverse effects of C1q are mediated by C5. We expect that the same mechanism applies to TBI. We have overcome problems with delays in obtaining and breeding these mice and, in the C1inhKO mouse strains, initiated the TBI studies. Although these animals have not been aged out, we are excited to already see brief transgene effects during recovery from TBI which quickly dissipate so that the health and behavior of the different strains become indistinguishable from each other, similar to humans who acutely show excellent recovery from mild TBI. The brief (<20 seconds) and subtle changes during recovery are that tau Tg mice and to a lesser extent the C1inhKO show an increase in Class I seizure-like symptoms, compared to control mice subjected to TBI. These studies are likely to identify key inflammatory mechanisms contributing to deficits after mild-TBI.

15. SUBJECT TERMS
Glia, microglia, mild traumatic brain Injury, chronic traumatic encephalopathy, complement cascade, neuroinflammation, neurofibrillary tangles, tau, trans-synaptic, phagocytosis

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1. **INTRODUCTION:** The purpose of this study is to use animal models to elucidate the mechanisms after repeated mild traumatic brain injury (mTBI), leading to neurodegenerative disease, such as chronic traumatic encephalopathy that occur after several asymptomatic months or years. This long asymptomatic period suggests that the brain has strong protective mechanisms against deleterious effects, but that eventually there is failure to compensate. The main pathology thought to cause onset of the disease is accumulation of abnormal aggregates of a protein called tau, which is a pathology common to many neurodegenerative diseases. Chronic aberrant neuroinflammation (dysregulation of astrocytes and microglia), during the asymptomatic period is known to drive tau pathogenesis through activating tau kinases, but the mechanisms remain elusive. We have identified an inflammatory pathway called the complement cascade involving the microglia, which plays an essential role in synaptic pruning, but no model to date has modeled its hyperactivation, known to occur after TBI. Since our preliminary data shows that C1q plays a prominent role in tau accumulation and that these effects are mediated by C5 convertase, we have obtained novel models that will for the first time allow study of these mechanisms. Our data also show that the complement cascade plays a role in tau accumulation that is distinctive and opposite from its role in amyloid accumulation. This proposal investigates the hypothesis that the dysregulation of glia plays a critical role in tau spreading leading to cognitive loss.
2. **KEYWORDS:** Microglia, Astrocytes, tau, complement 5a, serpin, C1 esterase inhibitor, tau kinases, chronic traumatic encephalopathy, post-traumatic brain injury, trans-synaptic, phagocytosis.
3. **ACCOMPLISHMENTS:**
   - **What were the major goals of the project during this Period (Year 1)?**
     - **The first major goal "Major Task 1"was to obtain** ACURO and Institutional Animal Approvals. We successfully achieved the milestone to obtain approval to initiate animal studies within the target 3 months (100% complete). This included obtaining ACURO (Subtask 1) and UCLA approval (Subtask 2). Although Subtask 3 was not part of the milestone we were unable to hire a post doctoral researcher by the target date of 3 months, and at this time (12 months) this is only 50% complete.
     - **The second major goal "Major Task 2"was to p**roduce mice for our Specific Aim 1, the GFAP C5a Tg cross (first generation heterozygous). The milestone to produce crosses, randomize and assign to treatments by 14 months is only 50% complete at this time (12 months). The target date to obtain mice from Dr. Tenner at UC Irvine (Subtask 1) was 4 months but we were not able to obtain these mice until 9 months. This task is 100%. Complete. This has led to a delay in the ability to breed mice to obtain four

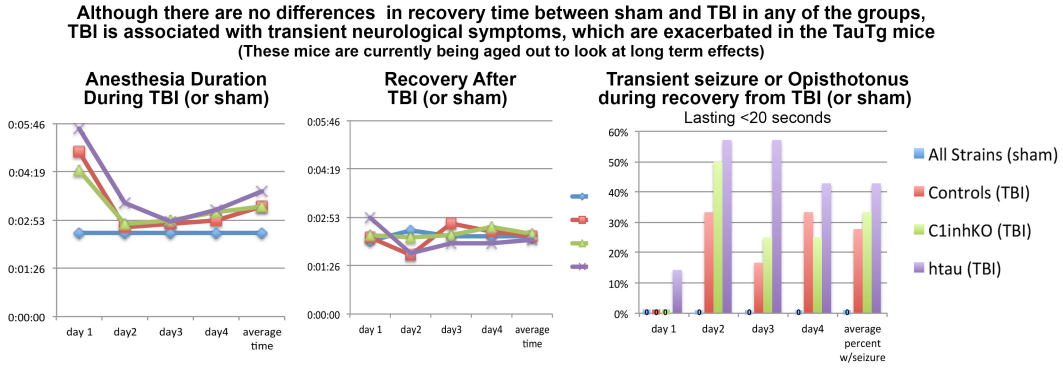
transgenes needed for the GFAP C5a Tg study. So although the target date was 12 months, this is only 50% complete at this time (12). Similarly mice have not been randomized for the study (subtask 3). The target date for this subtask was 14 months and is only 10 % complete since only 10% of the mice have been randomized at this time (12 months).

- **The third major goal "Major Task 3" was to** perform TBI (4x at 24 hr interval) on the randomized C5a mice (4 strains). Although the milestone was to complete behavioral tests by Target 25 months , one of the subtasks (subtask 1, Target **9-16** months) is **10 % complete** at this time (12 months), since some of the controls have already been subjected to TBI and we are aging them out.
- **The remaining goals relevant to year 1 period, related to the Major Goal 5, the C1inh KO studies (Major Task 5)**: The milestone for this goal to produce the mice was 13 months and it is (60% complete at 12 months). Subtask 1A (approval from Washington University to obtain the C1inhKO mice and Subtask 1B (receiving the C1inhKO mice) and Subtask 2 (breeding these mice with htau) are 100% complete. However Subtask 3 (Target 11-13 months) is only 5% complete at 12 months.

- **The subsequent goal is to Perform repeated mild TBI (4x at 24 hr interval) on theC1 inh mice, with the** target of 25 months, and the milestone to establish tissue repositories (blood, brain, synaptosomes, histology sections, dissected brain regions for biochemistry). This is only 5% complete at 12 months, as we have gotten farther along with this strain than the C5 strain. For subtask 1, we have randomize two of the litters of mice to the respective groups. The target date for this subtask is 16 months, which is 5% complete at 12 months.
- **What was accomplished under these goals?**

  i. *Major activities related were a-obtaining institutional and ACURO approval for animal use. b-obtaining and breeding the Serpin KO (C1 esterase Inh)d-obtaining and rederiving and breeding the C5a GFAP mice. d-crossing the serpin KO and htau mice e-crossing the C5a GFAP mice. f-producing htau with mouse tau background and f-beginning the TBI studies with the serpin KO. The specific objectives to be accomplished during this period were to obtain the C1inh and C5GFAP transgenic mice from the respective institutions and breed them the obtain the crosses needed for the study and begin the TBI.*

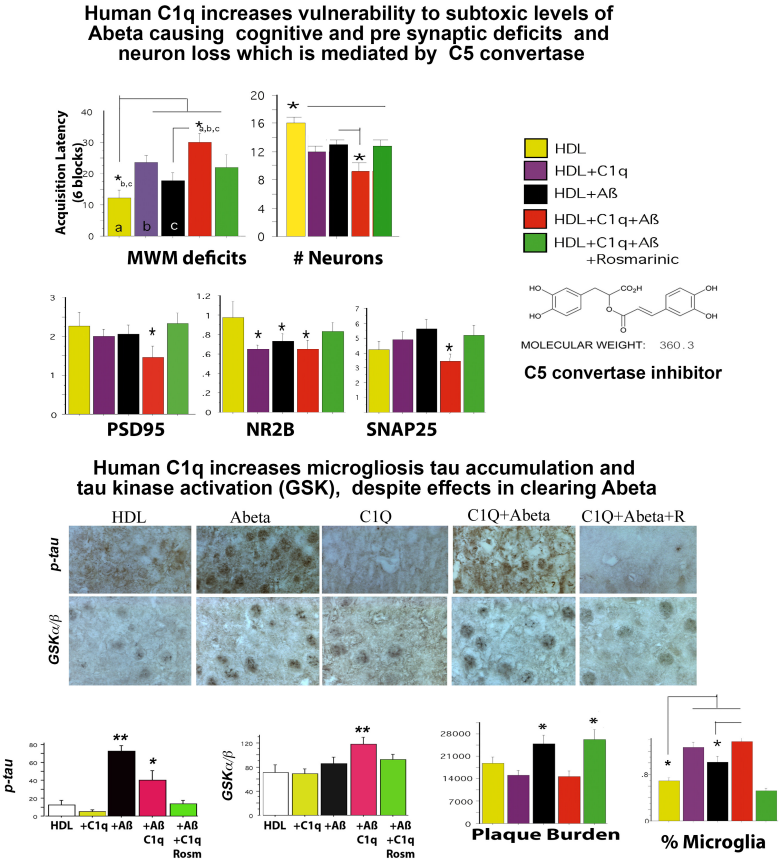
ii. *Significant results includes successful rederivation and viability of all the crosses. While the mice that have undergone TBI have not been aged out and euthanized, we identified differences in acute response to TBI. Unlike all the animals in all the groups subjected to TBI, none of the sham animals showed any Class I or II seizure or opisthotonus during recovery from TBI. The strain with the highest number of neurological responses, was the human Tau Tg and the C1inh KO mice showed a slight increase in Class I or II seizure or opisthotonus response to the TBI above control. Mice showing this transient neurological response recovered completely within 20 seconds, and appear healthy with no neurological symptoms as we are aging out .We expect this may predict a more severe pathology at onset of symptoms.*



iii. *Other achievements.*

1. *We have identified a plasma brain derived extracellular vesicle associated with gliosis in the brain in our human trial that response to anti-inflammatory drugs. Patients with mild cognitive deficits on the anti-inflammatory agent curcumin showed reduction in plasma extracellular vesicles containing GFAP (a glial marker). We are very excited about this since GFAP has been identified as a biomarker for TBI, but our assay is likely to improve sensitivity to neuroinflammation. We will be able to examine this in the plasma of TBI to determine its ability to predict brain inflammation.*

2. *Other achievements is the findings from examining C1q and C5a using tissues from a prior study. This strengthens the rationale for the study. We show that C1q increases the vulnerability to toxins (beta amyloid), particularly in the post synaptic proteins, and this is reversed by C5 convertase inhibition (rosmarinic acid), while some endpoints show direct toxic effects of C1q in the absence of toxins (presynaptic markers and gliosis). Further the fact that this pathway has a differential effect on amyloid burden, argues that the adverse effects of complement are mediated by tau accumulation, as opposed to the current dogma that Abeta binding to C1q has a pathogenic role, which extensive research continues to refute. The graph is shown here.*



iv. *Stated goals not met. One goal not met was not being able to hire a post-doctoral fellow. However we were able to replace the laboratory manager with a more experienced staff member (Peter Kim, SRA2) and Anna To (SRA). These staff are outstanding and have compensated for the lack of post doctoral fellow. The other goal we have not met is that we have not been able to breed the required number of mice due to delays in rederivation. We do not think this will impact the time course of the planned studies (except delays in producing the mice), as we are breeding more mice at once instead of staggering them the way that was planned, and have trained undergraduate students to assist in behavior.*

- **What opportunities for training and professional development has the project provided?**
  - We have trained undergraduate students (Ms. Anna To and Ms. Seungwong Jong "Sonny") under a research course (199). Anna has graduated and is now a highly skilled technician SRA1 in our

lab. We also have undergraduate work study students Nisha Choothakan. Other work study students are in training but have not yet begun working on this project.

- **How were the results disseminated to communities of interest?** *NOTHING TO REPORT*
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - *During the next reporting period, in order to accomplish the goals and objectives, we plan to breed more animals at once to keep on track for the three year period. That is although subtasks in production and testing of the mice will be delayed, we should be able to catch up by the third year with less staggering of mouse groups (producing more litters at a time). We have trained undergraduate students and are in the process of hiring the post doctoral fellow.*

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?**
     - The impact of the findings so far is that young mice with the human gene called 'tau' that causes the pathology associated with brain injury show subtle differences in acute responses to TBI, despite have no neurology or behavioral problems and this is exacerbated if they have a gene that stimulations the inflammatory pathway called "Complement Cascade". This demonstrates that high levels of tau are sufficient to cause mild problems in response to brain injury and that this is worsened by chronic inflammation. This subtle acute response is transient and the animals fully recover and adapt, but with time the eventual failure to adapt to chronic inflammation may lead to more severe problems.
   - **What was the impact on other disciplines?**
     - *This study may have an impact on understanding mechanisms of inflammation in other tauopathies (FTD, or Alzheimer's).*
     - *We have improved the techniques for TBI by creating a "helmut" to enable a closed skull injury give precise hits at the desired stereotaxic coordinates. We have controlled the hit velocity to ensure mice quickly recover, but have sufficient impact to show a slower recovery from anesthesia. We have found that a strike velocity of 5m/s, strike depth of 1.0mm, and dwell time of 200 milliseconds is optimal to ensure a quick and complete recovery but have sufficient force to show some mild and very transient neurological symptoms*
   - **What was the impact on technology transfer?** *NOTHING TO REPORT*
   - **What was the impact on society beyond science and technology?** *NOTHING TO REPORT*

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**. *NOTHING TO REPORT*
- **Actual or anticipated problems or delays and actions or plans to resolve them**
- *To fill the post doctoral fellow needed for Major Task 1 we posted the job at several local and national venues and interviewed the several candidates who responded to the posting. However, none were sufficiently knowledgeable about neuroinflammation and animal models, but eventually we were able to offer a PhD student with expertise in this field who is graduating this Fall for Ireland. The quality of our staff has compensated for not having a post doctoral fellow so we no longer foresee any problems related to staffing. Although we anticipate more serious problems with the difficulty in obtaining the required number of animals in the allotted time, which has caused some delays, we have a plan to resolve this. Problems included failures after repeatedly attempting to rederive embryos from C5a mice obtained from UC Irvine. We went through three embryo straws over 6 months, all which either died in utero or the mother failed to feed the pups or they were cannibalized. We finally got UCLA to agree to transfer a live breeding pair, which resolved our problem and they are successfully breeding and expanding our colony. We had similar problems with the C1inh mice, initially started by the male parent dying, requiring further crossing with wildtype then self- crossing the hemizygous obtain the homozygous. This delayed us 6 months after receiving the mice. In short, we have developed a plan to resolve obtaining the required number of mice of all of the strains. Previously our time frame allowed us to stagger litters, since it would be impossible to do all the animal behavior and euthanasia on so many litters at one. It is very labor intensive (since tissue has to be dissected and prepared for biochemistry and synaptosome preparation or fixed for histology at the time of euthanasia). We plan to breed more litters at one time so we can catch up, and have trained undergraduate work study students to assist in the labor intensive work with the animal behavior and euthanasia.*
- **Changes that had a significant impact on expenditures.** We have a carry over of $15,000 which will be used to pay work study students and animal per diem in the next year..
- **Significant changes in biohazards or select agents. N/A/**
- **Significant changes in use or care of human subjects. N/A**
- **Significant changes in use or care of vertebrate animals. NO**
- **Significant changes in use of biohazards and/or select agents. N/A**

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations** *NONE*
- **Journal publications.** *NONE*
- **Books or other non-periodical, one-time publications.** *NONE*
- **Other publications, conference papers, and presentations.** *NONE*
- **Website(s) or other Internet site(s)** *NONE*
- **Technologies or techniques**

  We are developing techniques to assess Plasma Extracellular Vesicles derived from the brain that may monitor inflammation related to TBI. Currently we are using human samples from another grant, and we can apply this new technology to the mouse models in this study. .
- **Inventions, patent applications, and/or licenses** *NONE*
- **Other Products**v*NONE*

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

Name:	*Peter Kim*
Project Role:	*Senior Research Associate 2*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*9*
Contribution to Project:	*Mr. Kim manages the colony and breeding and works with the PI to conduct the TBI. He genotypes mice and ensures that the appropriate number are bred for the DOD project and communicates weekly about progess. He is also responsible for overseeing the work of undergraduate students.*
Funding Support:	*N/A*

Name:	*Nicolae Kiosea (no longer an employee)*
Project Role:	*Senior Research Associate 2*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*3*
Contribution to Project:	*Mr. Kiosea was prior breeding colony manger an has now left the lab for another position in private biotech company*
Funding Support:	*N/A*

Name:	*Anna To*
Project Role:	*Senior Research Associate 1*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*3*
Contribution to Project:	*Ms. To assists the PI and Mr. Kim in euthanasia, dissections and behavior.*
Funding Support:	*N/A*

Name:	*Suengwong Jong ("Sonny")*
Project Role:	*Undergraduate student*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*3 (no cost)*
Contribution to Project:	*Ms. Wong is working on the project as an undergraduate 199 course. She is learning to conduct and analyze behavior*
Funding Support:	*N/A*

Name:	*Nisha Choothankan*
Project Role:	*Undergraduate student*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*3 (no cost)*
Contribution to Project:	*Ms. Choothankan working on the project learning to conduct biochemical analysis of brains. She has recently graduated in the lab and assigned to this project 50%.*
Funding Support:	*N/A*

Name:	*Edmond Teng*
Project Role:	*Associate Clinical Professor*
Researcher Identifier (e.g. ORCID ID):	*eteng2*
Nearest person month worked:	*1 (no cost)*
Contribution to Project:	*TBI methodology*
Funding Support:	*None*

Name:	*Andrea Tenner*
Project Role:	*Director of MIND institute UC Irvine*
Researcher Identifier (e.g. ORCID ID):	*andreatenner*
Nearest person month worked:	*1 (no cost)*
Contribution to Project:	*Provided C5a Tg mice and assisting in recovering embryos and troubleshooting rederivation of the colony at UCLA*
Funding Support:	*T32 AG000096 "Training in the Neurobiology of Aging"* NIH NIA (PI, C.W. Cotman, Project Leader - A.J. Tenner) 5-01-14 through 4-30-19 $250,000 P01 AG 00538 "Behavioral and Neural Plasticity in the Aged", Project Neuroprotection and neuroinflammation induced by complement proteins and receptors $800,000 5-01-14 through 4-30-19

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
- *Yes I have received an NIH R21 award to study ApoE isotype effect on tau pathology. Also I have been awarded a new VA MERIT on a mixed model of dementia using transgenic rats with spontaneously hypertensive background.*
  - R21AG050269 Modulation of tau pathogenesis by high dietary fat, gender and ApoE isoform. July 1, 2016 to June 30, 2018
  - BX3485003485 Neuroinflammation and Neurodegeneration in a Transgenic Alzheimer Rat with Vascular Disease November 1, 2016 to October 31, 2020
- **What other organizations were involved as partners?**
  - *N/A*
- **Personnel exchanges**
  - *N/A*

8. **SPECIAL REPORTING REQUIREMENTS**

- **COLLABORATIVE AWARDS:** *N/A*
- **QUAD CHARTS:** *N/A*

9. **APPENDICES:** *NO APPENDICES.*